FIG. 11

Signal to noise ratio of ELISA reading using phospho-Serine/threonine phenylalanine antibody.

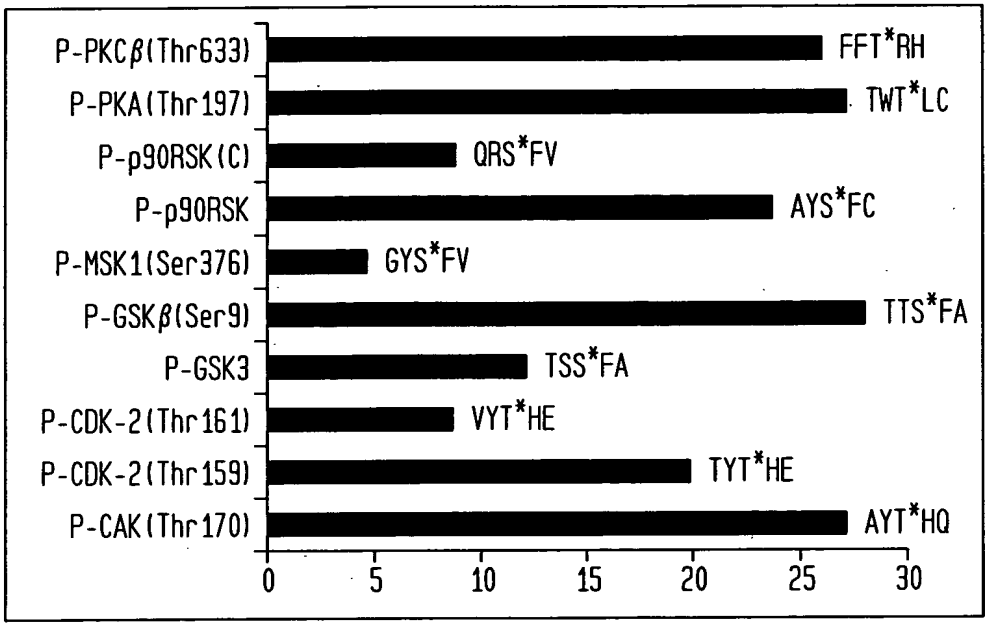
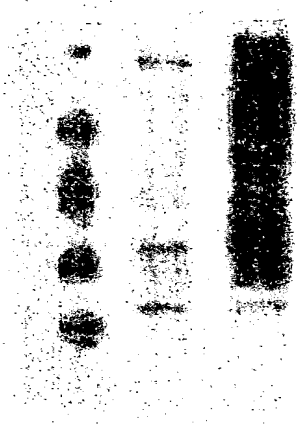


FIG. 12

Western analysis of calyculin A-treated A431 cells using phospho-Serine/phenylalanine substrates antibody.



\- +
calyculin A

BEST AVAILABLE

FIG. 7

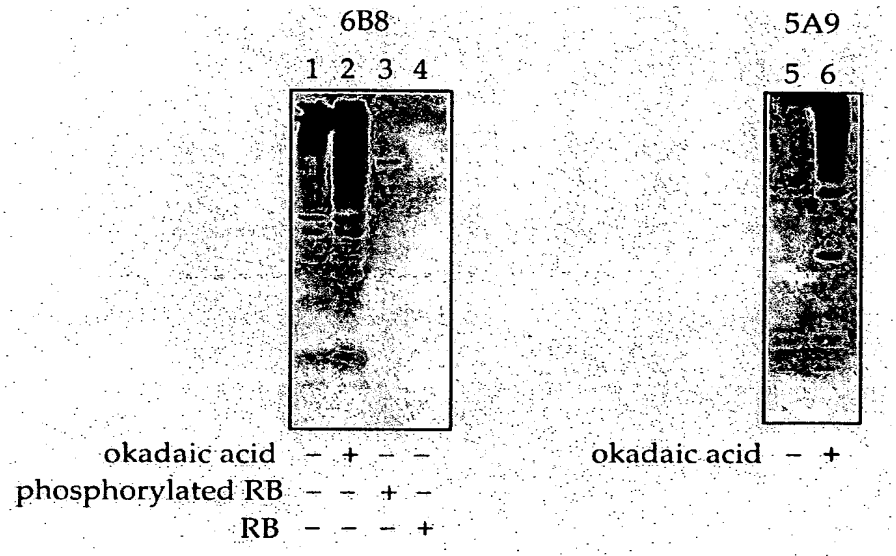
Western analysis of calyculin A-treated A431 cells using Phospho-Akt Substrate Antibody.

\- +

calyculin A

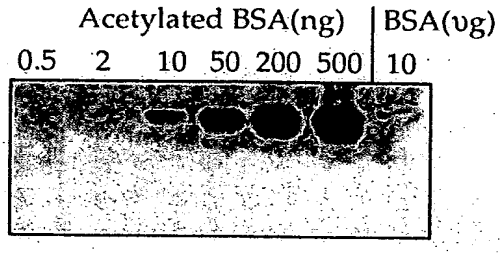
BEST AVAILABLE COPY

FIG. 4B



BEST AVAILABLE COPY

FIG. 5A



BEST AVAILABLE COPY

FIG. 5B

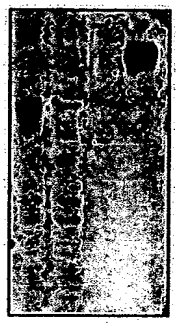


FIG. 5C

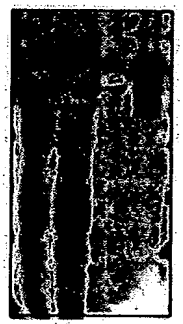


FIG. 5D

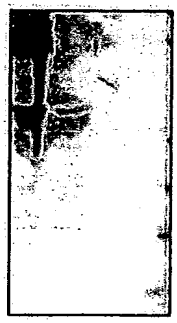


FIG. 3B

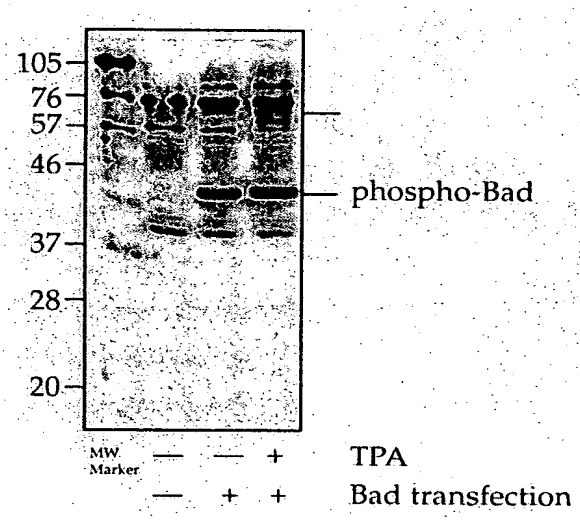
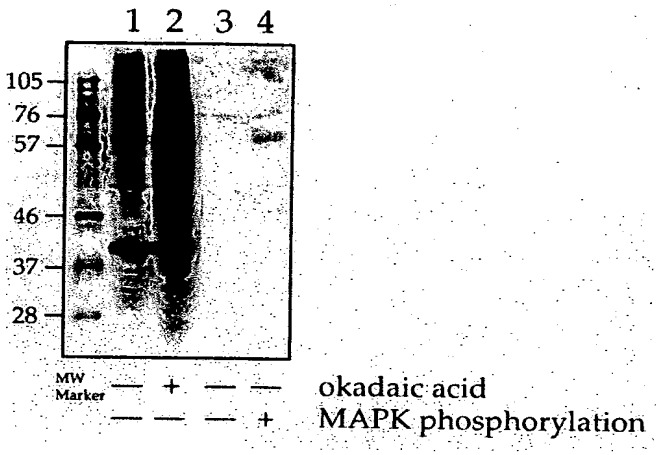

105
76
57
46
37
28
20
phospho-Bad
MW Marker
— — +
TPA
— + +
Bad transfection

FIG. 2B


1 2 3 4
105
76
57
46
37
28
MW Marker
— + — — okadaic acid
— — — + MAPK phosphorylation

- + calyculin A

FIG. 9

Western analysis of calyculin A-treated A431 cells using Phospho-PKA Substrate Antibody.

BEST AVAILABLE COPY

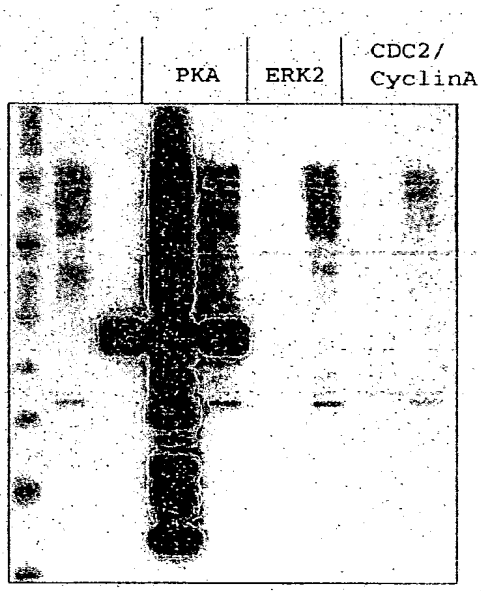


FIG. 10

Western analysis of A431 cell extracts

OIPE JAN 28 2005 PATENT & TRADEMARK OFFICE JC79

FIG. 1A

				ANTIBODY DILUTIONS				
PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
Thr*	X-X-X-X-X-Thr*-X-X-X-X-X-X-Cys	1.92	1.32	0.54	0.34	0.07	0.04	0.02
Ser-Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.11	0.05	0.01	0.00	0.01	0.00	0.00
Threonine* mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.26	0.10	0.07	0.05
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01	0.00
Akt-Thr308-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr*-Phe-Cys-Gly-Thr-Pro	1.18	0.65	0.24	0.13	0.03	0.01	0.00
	(SEQ ID NO:1)							
APP1-Thr668-P	Asp-Ala-Ala-Val-Thr*-Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys	0.14	0.03	0.01	0.01	0.01	0.01	0.00
	(SEQ ID NO:2)							
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Asn-Thr*-Phe-Val-Gly-Thr-Pro-Phe-Cys	1.71	1.13	0.39	0.22	0.03	0.02	0.02
	(SEQ ID NO:3)							
CAK-Thr167-P	His-Gln-Val-Val-Thr*-Arg-Trp-Tyr-Arg-Cys	1.77	1.15	0.41	0.27	0.06	0.03	0.01
	(SEQ ID NO:4)							
CAMIV-Thr196-P	His-Gln-Val-Leu-Met-Lys-Thr*-Val-Cys-Gly	1.79	1.36	0.63	0.40	0.09	0.05	0.01
	(SEQ ID NO:5)							
CDC2-Thr167-P	Ile-Pro-Ile-Arg-Val-Tyr-Thr*-His-Glu-Val-Val-Thr-Leu-Cys	1.02	0.56	0.14	0.08	0.03	0.01	0.01
	(SEQ ID NO:6)							
CDK2-Thr159-P	Gly-Val-Pro-Val-Arg-Thr-Tyr-Thr*-His-Glu-Val-Val-Thr-Leu-Cys	1.88	1.79	0.51	0.44	0.08	0.04	0.02
	(SEQ ID NO:7)							
p70S6K-Thr389-P	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr*-Tyr-Val-Ala-Pro-Lys-Lys-Cys	1.99	1.44	0.62	0.39	0.08	0.04	0.01
	(SEQ ID NO:8)							
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr*-Phe-Cys	1.82	1.63	0.94	0.58	0.15	0.08	0.02
	(SEQ ID NO:9)							
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr*-Glu-Tyr*-Val-Ala-Thr-Arg-Trp-Cys	1.56	1.18	0.51	0.30	0.07	0.04	0.02
	(SEQ ID NO:10)							
Myc Ser58/62-P	Glu-Leu-Leu-Pro-Thr*-Pro-Pro-Leu-Ser*-Pro-Ser-Arg-Arg-Ser-Cys	0.11	0.05	0.03	0.02	0.02	0.02	0.02
	(SEQ ID NO:11)							
P38-2P	Leu-Ala-Arg-His-Thr-Asp-Asp-Glu-Met-Thr*-Gly-Tyr*-Val-Ala-Thr-Arg-Cys	0.54	0.30	0.08	0.08	0.04	0.04	0.02
	(SEQ ID NO:12)							
JNK-2P	Ser-Phe-Met-Met-Thr*-Pro-Tyr*-Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys	1.49	0.44	0.12	0.07	0.03	0.02	0.02
	(SEQ ID NO:13)							

FIG. 1B

PEPTIDE SEQUENCE	phospho-Thr Reactivity
XXXXXS*XXXXX	—
XXXXY*XXXX	—
XXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	+ +
XXXXRSXS*XPXXXX (SEQ ID NO:15)	—
XXXXRSXSXPXXXX (SEQ ID NO:16)	—
XXXXPXS*/T*PXXXXX (SEQ ID NO:17)	+ +
XXXXPXS/TPXXXXX (SEQ ID NO:18)	—
XXXXT*XXXXXX	+ + +
XXXXXS/TXXXXXX	—
21 phospho-Thr peptides mixture	+ + +
38 phospho-Ser peptides mixture	—
30 phospho-Tyr peptides mixture	—
NEB LIBRARY	
X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19)	+ + +
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	+ +
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+ + +
X-X-X-X-R/K-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+ + +
X-X-X-R/K-X-X-S*/T*X-X-X-X-X-X-C (SEQ ID NO:23)	+ + +
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+ + +
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-C (SEQ ID NO:25)	+ + +
X-X-X-X-X-X-S*/T-P-X-X-X-X-X-X-C (SEQ ID NO:26)	+ +
X-X-X-X-X-T*-X-X-X-X-X-X-C	+ + +
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27)	+ +
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-C (SEQ ID NO:29)	+ +

ANTIBODY REACTIVITY		*ELISA O.D.*
+ + +	*very strong*	*> 2*
+ +	*strong*	*1 – 2*
+	*weak*	*0.2 - 1*
–	*very little*	*< 0.2*

FIG. 1D

Fixed Amino Acid	–5–4–3–2–1 +1+2+3+4+5 X X X X X Ser*/Thr* X X X X X Fixed AA position relative to phospho-Ser*/Thr*							
	– 4	– 3	– 2	– 1	S*/T*	+ 1	+ 2	+ 3
Ala	+ +	+ +	+ + +	+ + +		+ + +	+ + +	+ +
Cys	+ + +	+ + +	+ + +	+ + +		+ + +	+ + +	+ +
Asp	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Glu	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Phe	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Gly	+ +	+ +	+ +	+ +		+ + +	+ + +	+ +
His	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Ile	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Lys	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Leu	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Met	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Asn	+ +	+ + +	+ +	+ + +		+ + +	+ + +	+ +
Pro	+ +	+ +	+ +	+ + +		—	+ + +	+ +
Gln	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Arg	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Ser	+ +	+ +	+ +	+ +		+ + +	+ + +	+ +
Thr	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Val	+ +	+ +	+ +	+ + +		+ + +	+ + +	+ +
Trp	+ +	+ +	+ +	+ + +		+ + +	+ + +	+
Tyr	+ +	+ +	+ +	+ + +		+ +	+ + +	+

FIG. 2A

				ANTIBODY DILUTIONS				
PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
PXSP-P	X-X-X-X-X-Pro-X-Ser*-Thr*-Pro-X-X-X-X-X-Cys	1.82	1.97	1.74	1.40	0.70	0.35	0.08
	(SEQ ID NO:27)							
Threonine mix	18 phospho-Thr peptide mix	1.97	1.37	0.67	0.36	0.13	0.07	0.05
Ser/Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.14	0.03	0.01	0.00	0.00	0.00	0.00
	(SEQ ID NO:28)							
RB Thr373-P	Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys	2.07	2.17	1.70	1.20	0.48	0.18	0.03
	(SEQ ID NO:30)							
MKK3-Thr-P	Ser-Val-Ala-Lys-Thr*-Met-Asp-Ala-Gly-Cys	0.06	0.04	0.01	0.00	0.00	0.00	0.00
	(SEQ ID NO:31)							
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr*-Phe-Cys	0.05	0.02	0.01	0.00	0.01	0.00	0.00
	(SEQ ID NO:9)							
p70 S6K-Thr389	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr*-Tyr-Val-Ala-Pro-Lys-Lys-Cys	0.11	0.05	0.01	0.00	0.01	0.00	0.00
	(SEQ ID NO:8)							
cdk4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr*-Pro-Val-Val-Val-Lys-Cys	2.07	2.21	2.01	1.55	0.69	0.31	0.07
	(SEQ ID NO:32)							

FIG. 3A

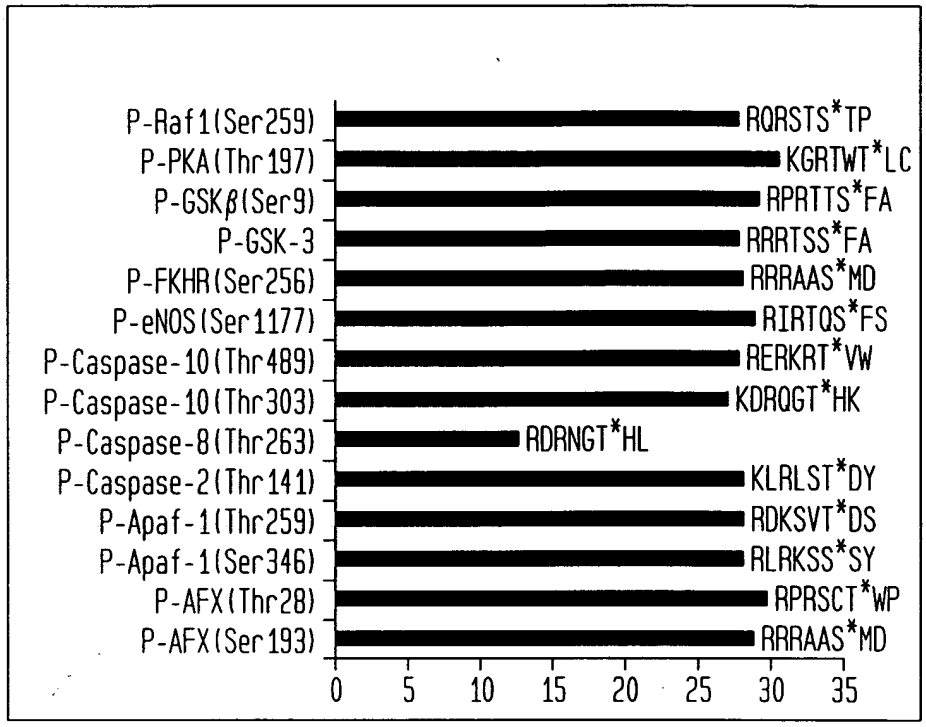
				ANTIBODY DILUTIONS			
PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05
14-3-3 BM-P	X-X-X-X-Arg-Ser-X-Ser*-X-Pro-X-X-X-X-Cys	2.41	2.15	1.49	1.15	0.44	0.25
	(SEQ ID NO:33)						
14-3-3 BM	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-X-Cys	0.07	0.03	0.02	0.03	0.02	0.03
	(SEQ ID NO:34)						
CDC25-Ser216-P	Gly-Leu-Tyr-Arg-Ser-Pro-Ser*-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys	2.35	2.08	1.49	1.05	0.33	0.18
	(SEQ ID NO:35)						
CDC25-Ser216	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys	0.05	0.02	0.03	0.03	0.04	0.03
	(SEQ ID NO:36)						
Bad-Ser112-P	Thr-Arg-Ser-Arg-His-Ser-Ser*-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys	1.59	0.43	0.10	0.03	0.01	0.00
	(SEQ ID NO:37)						
Bad-Ser112	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys	0.00	0.00	0.00	0.00	0.00	0.00
	(SEQ ID NO:38)						
Bad-Ser136	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys	0.03	0.00	0.00	0.00	0.00	0.00
	(SEQ ID NO:39)						
Bad-Ser136-P	Phe-Arg-Gly-Arg-Ser-Arg-Ser*-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys	3.25	1.86	0.73	0.51	0.07	0.03
	(SEQ ID NO:40)						

FIG. 4A

		MONOCLONAL ANTIBODIES	
PEPTIDE	SEQUENCE	6B8	5A9
Ser/ThrPro-P	X-X-X-X-X-X-Ser*/Thr-Pro-X-X-X-X-X-X-Cys	1.774	0.731
	(SEQ ID NO:26)		
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser*/Thr*-Pro-X-X-X-X-X-Cys	0.924	0.766
	(SEQ ID NO:27)		
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys	0.02	0.063
	(SEQ ID NO:41)		
ProXSer/ThrProXArg-P	X-X-X-X-X-Pro-X-Ser*/Thr*-Pro-X-Arg/Lys-X-X-X-Cys	1.955	1.275
	(SEQ ID NO:42)		
Thr-P	X-X-X-X-X-X-Thr*-X-X-X-X-X-X-Cys	0	--
Ser-P	X-X-X-X-X-X-Ser*-X-X-X-X-X-X-Cys	0.031	0.088
Ser/Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.021	0.066
Tyr-P	X-X-X-X-X-X-Tyr*-X-X-X-X-X-X-Cys	0.023	0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-Ile-Pro-Gly-Cys	0.032	0.124
	(SEQ ID NO:43)		
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys	3.336	3.503
	(SEQ ID NO:30)		
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys	0.02	0.073
	(SEQ ID NO:44)		

FIG. 6

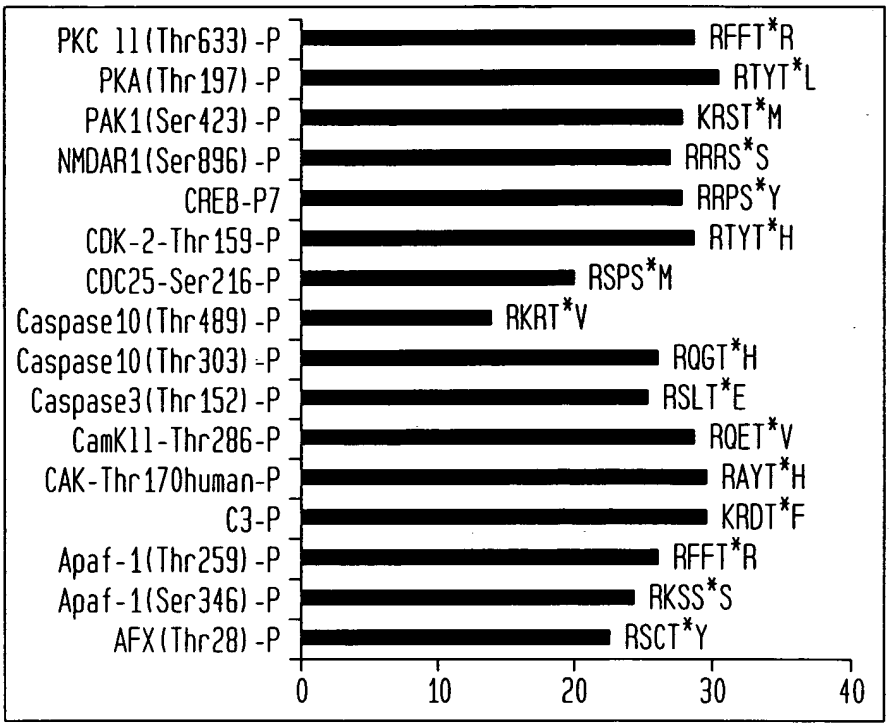
Signal to noise ratio of ELISA readings using phospho-Akt substrate antibody.



(SEQ ID NO:48)
(SEQ ID NO:49)
(SEQ ID NO:50)
(SEQ ID NO:51)
(SEQ ID NO:52)
(SEQ ID NO:53)
(SEQ ID NO:54)
(SEQ ID NO:55)
(SEQ ID NO:56)
(SEQ ID NO:57)
(SEQ ID NO:58)
(SEQ ID NO:59)
(SEQ ID NO:60)
(SEQ ID NO:61)

FIG. 8

Signal to noise ratio of ELISA reading using phospho-PKA substrates antibody.



(SEQ ID NO:62)
(SEQ ID NO:63)
(SEQ ID NO:64)
(SEQ ID NO:65)
(SEQ ID NO:66)
(SEQ ID NO:67)
(SEQ ID NO:68)
(SEQ ID NO:69)
(SEQ ID NO:70)
(SEQ ID NO:71)
(SEQ ID NO:72)
(SEQ ID NO:73)
(SEQ ID NO:74)
(SEQ ID NO:75)
(SEQ ID NO:76)
(SEQ ID NO:77)

OIPE JAN 28 2005 PATENT & TRADEMARK OFFICE JC79

FIG. 1A

				ANTIBODY DILUTIONS				
PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
Thr*	X-X-X-X-X-Thr*-X-X-X-X-X-X-Cys	1.92	1.32	0.54	0.34	0.07	0.04	0.02
Ser-Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.11	0.05	0.01	0.00	0.01	0.00	0.00
Threonine* mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.26	0.10	0.07	0.05
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01	0.00
Akt-Thr308-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr*-Phe-Cys-Gly-Thr-Pro	1.18	0.65	0.24	0.13	0.03	0.01	0.00
	(SEQ ID NO:1)							
APP1-Thr668-P	Asp-Ala-Ala-Val-Thr*-Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys	0.14	0.03	0.01	0.01	0.01	0.01	0.00
	(SEQ ID NO:2)							
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Asn-Thr*-Phe-Val-Gly-Thr-Pro-Phe-Cys	1.71	1.13	0.39	0.22	0.03	0.02	0.02
	(SEQ ID NO:3)							
CAK-Thr167-P	His-Gln-Val-Val-Thr*-Arg-Trp-Tyr-Arg-Cys	1.77	1.15	0.41	0.27	0.06	0.03	0.01
	(SEQ ID NO:4)							
CAMIV-Thr186-P	His-Gln-Val-Leu-Met-Lys-Thr*-Val-Cys-Gly	1.79	1.36	0.63	0.40	0.09	0.05	0.01
	(SEQ ID NO:5)							
CDC2-Thr167-P	Ile-Pro-Ile-Arg-Val-Tyr-Thr*-His-Glu-Val-Val-Thr-Leu-Cys	1.02	0.56	0.14	0.08	0.03	0.01	0.01
	(SEQ ID NO:6)							
CDK2-Thr159-P	Gly-Val-Pro-Val-Arg-Thr-Tyr-Thr*-His-Glu-Val-Val-Thr-Leu-Cys	1.88	1.79	0.51	0.44	0.08	0.04	0.02
	(SEQ ID NO:7)							
p70S6K-Thr389-P	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr*-Tyr-Val-Ala-Pro-Lys-Lys-Cys	1.99	1.44	0.62	0.39	0.08	0.04	0.01
	(SEQ ID NO:8)							
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr*-Phe-Cys	1.82	1.63	0.94	0.58	0.15	0.08	0.02
	(SEQ ID NO:9)							
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr*-Glu-Tyr*-Val-Ala-Thr-Arg-Trp-Cys	1.56	1.18	0.51	0.30	0.07	0.04	0.02
	(SEQ ID NO:10)							
Myc Ser58/62-P	Glu-Leu-Leu-Pro-Thr*-Pro-Pro-Leu-Ser*-Pro-Ser-Arg-Arg-Ser-Cys	0.11	0.05	0.03	0.02	0.02	0.02	0.02
	(SEQ ID NO:11)							
P38-2P	Leu-Ala-Arg-His-Thr-Asp-Asp-Glu-Met-Thr*-Gly-Tyr*-Val-Ala-Thr-Arg-Cys	0.54	0.30	0.08	0.08	0.04	0.04	0.02
	(SEQ ID NO:12)							
JNK-2P	Ser-Phe-Met-Met-Thr*-Pro-Tyr*-Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys	1.49	0.44	0.12	0.07	0.03	0.02	0.02
	(SEQ ID NO:13)							